BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 15/62, C07K 14/705, A61K 38/17

A1

(11) International Publication Number: **WO 98/49305**

(43) International Publication Date: 5 November 1998 (05.11.98)

(21) International Application Number: PCT/US98/08631

(22) International Filing Date: 29 April 1998 (29.04.98)

(30) Priority Data:
08/850,188 1 May 1997 (01.05.97) US

(71) Applicant *(for all designated States except US)*: AMGEN INC. [US/US]; Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320–1789 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BOYLE, William, J. [US/US]; 11678 Chestnut Ridge Street, Moorpark, CA 93021 (US). WOODEN, Scott [US/US]; 1668 Sweet Briar Place, Thousand Oaks, CA 91362 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320–1789 (US).

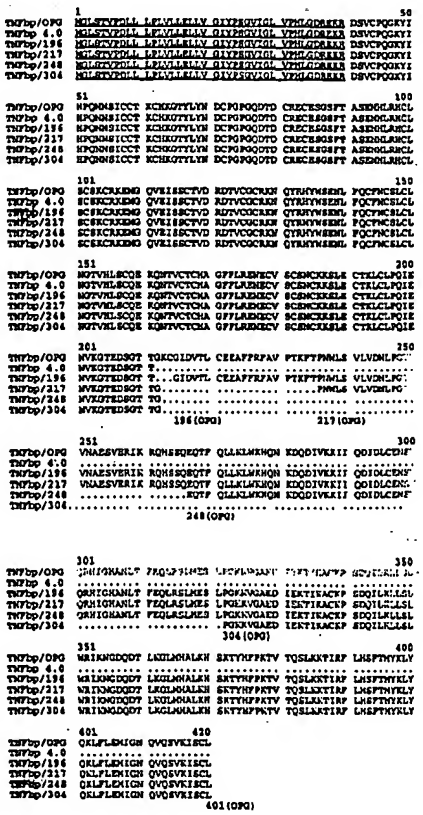
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHIMERIC OPG POLYPEPTIDES

(57) Abstract

Chimeric polypeptides comprising fusions of an osteoprotegerin dimerization domain to a heterologous sequence are provided. Also provided are nucleic acids encoding the polypeptides, expression vectors and host cells for their production and pharmaceutical compositions comprising the polypeptides.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

CHIMERIC OPG POLYPEPTIDES

Field of the Invention

The invention relates generally to chimeric polypeptides. More particularly, the invention relates to chimeric polypeptides comprising a fusion of an osteoprotegerin dimerization domain to a heterologous sequence. The polypeptides may be used in a variety of diagnostic and therapeutic applications.

Background of the Invention

Cells recognize a variety of signals which modulate growth, differentiation and metabolism. Effectors of cellular functions include small molecular weight organic compounds, carbohydrates, amino acids, peptides and proteins. At present, the best understood signalling process employs secretion of a signalling molecule from one cell to modulate functions of other cells (autocrine regulation). It has also been observed that secreted signalling molecules may also modulate the functions of cells which secrete them (paracrine regulation). The ability of cells to respond to external signals usually requires that the appropriate receptors which bind the signalling molecules be present on the cell surface. Protein-mediated signalling between cells involves binding of growth factors, hormones, cytokines, cell adhesion proteins and the like to cell surface receptors.

As a class of proteins, receptors vary in their structure and mode of signal transduction. They are characterized by having an extracellular domain that is involved in binding a signalling molecule and

cytoplasmic domain which transmits an appropriate intracellular signal. Receptor expression patterns ultimately determine which cells will respond to a given ligand, while the structure of a given receptor dictates the cellular response induced by ligand binding. Receptors have been shown to transmit intracellular signals via their cytoplasmic domains by activating protein tyrosine, or protein serine/threonine phosphorylation (e.g., platelet derived growth factor receptor (PDGFR) or transforming growth factor-β receptor-I (TGFβR-I), by stimulating G-protein activation (e.g., β-adrenergic receptor), and by modulating associations with cytoplasmic signal transducing proteins (e.g., TNFR-1 and Fas/APO) (Heldin, Cell 80, 213-223 (1995)).

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I transmembrane proteins which share a conserved cysteine-rich motif which is repeated three to six times in the extracellular domain (Smith, et al. Cell 76, 953-962 (1994)). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., Chemistry 270, 2874-2878 (1995)). The ligands for these receptors are a structurally related group of proteins homologous to TNFα. (Goeddel et al. Cold Spring Harbor Symp. Quart. Biol. 51, 597-609 (1986); Nagata et al. Science 267, 1449-1456 (1995)). TNFα binds to distinct, but closely related receptors, TNFR-1 and TNFR-2. TNFα produces a variety of biological responses in receptor bearing cells, including, proliferation, differentiation, and cytotoxicity and apoptosis (Beutler et al. Ann. Rev. Biochem. 57, 505-518 (1988)).

TNFα is believed to mediate acute and chronic inflammatory responses (Beutler et al. ibid). Systemic delivery of TNFα induces septic shock-like syndrome and

widespread tissue necrosis. Because of this, TNFα may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis. Mutations in FasL, the ligand for the TNFR-related receptor Fas/APO (Suda et al. Cell 75, 1169-1178 (1993)), is associated with autoimmunity (Fisher et al. Cell 81, 935-946 (1995)), while overproduction of FasL may be implicated in drug-induced hepatitis. Thus, ligands to the various TNFR-related proteins often mediate the serious effects of many disease states, which suggests that agents that neutralize the activity of these ligands would have therapeutic value.

Soluble TNFR-1 receptors and antibodies that bind TNFα have been tested for their ability to neutralize systemic TNFα (Loetscher et al. Cancer Cells 3, 221-226 (1991)). A naturally occuring form of a secreted TNFR-1 and TNFR-2 mRNA was recently cloned, and its product tested for its ability to neutralize TNFα activity in vitro and in vivo (Kohno et al. Proc. Natl. Acad. Sci. USA 87, 8331-8335 (1990)). The ability of this protein to neutralize TNFα suggests that soluble TNF receptors function to bind and clear TNF thereby blocking the cytotoxic effects on TNFR-bearing cells.

Recombinantly-produced TNF inhibitors have also been taught in the art. For example, EP 393 438 and EP 422 339 teach the amino acid and nucleic acid sequences of a "30kDa TNF inhibitor" (also known as a p55 receptor) and a "40kDa inhibitor" (also known as a p75 receptor) as well as modified forms thereof, e.g., fragments, functional derivatives and variants. EP 393 438 and EP 422 339 also disclose methods for isolating the genes responsible for coding the

inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors. Mature recombinant 30kDa TNF inhibitor and mature recombinant 40kDa TNF inhibitor have been demonstrated to be capable of inhibiting TNF (EP 393 438, EP 422 339, PCT Publication No. WO 92/16221 and PCT Publication No. WO 95/34326).

A recently identified member of the TNFR family, termed Osteoprotegerin (OPG), is a secreted polypeptide which inhibits osteoclast maturation and markedly increases bone density in transgenic mice expressing the OPG polypeptide. OPG inhibited in vitro the formation of mature osteoclasts from hematopoietic progenitor cells and reduced the extent of bone loss in ovariectomized rats (see co-owned and co-pending U.S. Serial Nos. 08/577,788, filed December 22, 1995; 08/706,945, filed September 3, 1996; and 08/771,777 filed December 20, 1996). OPG may have benefit in the treatment of osteopenia. PCT Application No. WO96/26217 discloses a polypeptide termed Osteoclastogenesis Inhibitory Factor (OCIF) which is identical to OPG.

OPG comprises two domains having different structural and functional properties. The amino-terminal domain spanning residues 22-194 in the mature polypeptide shows homology to other members of the TNFR family, especially TNFR-2, through conservation of cysteine rich domains characteristic of TNFR family members. The carboxy terminal domain spanning residues 194-401 has no significant homology to any known sequences. Unlike a number of other TNFR family members, OPG appears to be exclusively a secreted protein and does not appear to be synthesized as a membrane associated form. Analysis of OPG by reducing and non-reducing gel electrophoresis indicated that the full-length mature polypeptide of 380 amino

acids formed a dimer having a molecular weight of about 120 kDa as compared to the monomer molecular weight of about 60 kDa. OPG polypeptides having certain truncations in the carboxy terminal domain or substitutions of certain cysteine residues within in the carboxy terminal domain formed dimeric OPG to a lesser extent and had lower biological activity compared to wild-type OPG. However, replacement of part or all of the OPG carboxy terminal domain with an Fc region of IgG restored biological activity in the OPG fusion protein to near normal levels. Based upon these observations, the amino-terminal region of OPG appeared to be required for biological activity while the carboxy-terminal domain was important for dimerization. In addition, the biological activity of OPG appeared to be enhanced when the molecule was in dimeric form.

In a therapeutic regimen, it is often desirable to modulate a biological response either by enhancing or blocking a signal received by a receptor. Enhancement of a biological response can involve increasing the affinity of the signalling molecule for a receptor, or increasing the half-life of the molecule in circulation such that it is bound to the receptor for a longer period of time. When the signalling molecule is a polypeptide, enhancement of a biological response may be achieved by constructing analogs which have amino acid sequence changes that increase binding or half-life, derivatives (e.g., polypeptides modified with water soluble polymers) to increase solubility and/or half-life, or chimeric polypeptides (e.g, polypeptides fused to the Fc region of IgG) which increase half-life, solublility and/or modify the aggregation state of the protein in circulation. Similar approaches may be taken to develop therapeutic proteins which act as antagonists by blocking a

biological response. In particular, soluble forms of transmembrane receptors which may encompass part or all of the extracellular domains have been used to prevent ligand binding and receptor activation. Soluble receptors have been developed as chemically-modified derivatives and as chimeric polypeptides.

Due to the relatively low inhibition of cytotoxicity exhibited by the 30kDa TNF inhibitor and 40kDa TNF inhibitor (Butler et al. Cytokine 6, 616-623 (1994)), various groups have generated dimers of TNF inhibitor proteins (Butler et al. (1994), supra; and Martin et al. Exp. Neurol. 131, 221-228 (1995)). However, the dimers may generate an antibody response (Martin et al. (1995), supra; and Fisher et al. New Eng. J. Med., 334, 1697-1702 (1996)).

Generation of chimeric polypeptides has been described in the art. For example, construction of hydrid immunoglobulin molecules by fusion of a ligand binding partner to a human IgG chain is described in U.S. Patent Nos. 5,116,964 and 5, 428,130. Construction of a chimeric polypeptide comprising the extracellular domain of a TNF receptor fused to a mouse IgG heavy chain is described in U.S. Patent No. 5,447,851. Chimeric polypeptides comprising the extracellular domain of a human PDGF receptor fused to dimerizing proteins is described in EP 0 721 983. Multimers of soluble forms of TNF receptors are described in U.S. Patent No. 5,478,925.

While fusion proteins, such as those comprising immunoglobulin constant regions, may have desirable biological properties, they can elicit an immune response which limits their usefulness as a human therapeutic.

Therefore, it is an object of the invention to provide chimeric polypeptides which enhance or block a biological response. Such polypeptides may have

increased stability, solubility, circulating half-life and decreased immunogenicity.

It is another object of the invention to provide chimeric polypeptides which combine the active region of a signalling molecule with an OPG dimerization domain wherein said chimeric polypeptides will enhance or block a biological response characteristic of the signalling molecule portion of the chimera.

It is another object of the invention to provide OPG chimeric polypeptides which form dimers, trimers and higher multimers which may have advantageous properties such as increased binding affinity, greater stability, and longer circulating half-life compared to monomeric forms.

Summary of the Invention

The invention provides for chimeric polypeptides comprising fusions of an OPG dimerization domain to a heterologous sequence. Also provided for are nucleic acid sequences encoding the polypeptides, expression vectors and host cells for production of the polypeptides, and pharmaceutical compositions comprising the polypeptides.

A heterologous sequence of the invention comprises an amino acid sequence of a cell signalling molecule, such as a receptor, an extracellular domain thereof, and an active fragment, derivative and analog of a receptor or an extraceullular domain. In a preferred embodiment, heterologous sequences are selected from the family of TNF-like receptors. Such sequences preferentially include functional extracellular ligand binding domains and lack functional transmembrane and cytoplasmic domains. In another embodiment, the transmembrane and cytoplasmic domains are deleted in whole or in part. It is

understood that heterologous sequences of the invention do not include the amino terminal region of OPG defined by residues 22-194 as shown in U.S. Serial No. 08/577,788 filed December 22, 1995 and hereby incorporated by reference, and do not include related amino acid sequences which, when fused to an OPG dimerization domain, exhibit the biological activity of OPG.

Also encompassed by the invention are multimeric polypeptides comprising covalently associated monomers of OPG chimeric polypeptides. The monomers may have identical heterolgous sequences or different heterologous sequences. In a preferred embodiment, the multimeric polypeptide is a dimer, either a heterodimer (different heterologous sequences) or a homodimer (identical heterologous sequences).

The chimeric polypeptides of the invention are produced by transforming or transfecting host cells with nucleic acids encoding the polypeptide, culturing the host cells, and recovering the polypeptide from the culture. Also provided for are expression vectors and host cells for producing the chimeric polypeptides.

The chimeras are useful for detecting molecules which interact with fused heterologous sequences and thereby identifying potential new receptors and ligands. The compositions of chimeric polypeptides provided herein are useful for treatment of a variety of disorders, for example those related to receptor binding. In one embodiment, compositions comprising TNF/OPG and TNFR/OPG chimeric are used to treat TNF and TNFR mediated disorders, such as inflammation, autoimmune diseases, and disorders related to excessive apoptosis

Description of the Figures

Figure 1. Amino acid sequences of human, mouse and rat OPG dimerization domains (residues 194-401 of corresponding full-length OPG polypeptides). Conserved cysteine residues implicated in disulfide bond formation are underlined.

Figure 2. Nucleic acid and amino acid sequence of mature, full-length 30 kDa TNF inhibitor.

Figure 3. Nucleic acid and amino acid sequence of mature, full-length 40 kDa TNF inhibitor.

Figure 4. Amino acid sequences of TNFbp/OPG chimeric polypeptides. The TNFbp portion of the chimera is the full-length 30 kDa TNF inhibitor with the leader sequence (underlined) and the additional sequence VKGTEDSGTT at the carboxy terminus. OPG dimerization domains are human OPG residues 194-401, 196-401, 217-401, 248-401 and 304-401. The junction of the TNFbp and OPG sequences creates an Age I restriction site in the DNA sequence and adds a glycine codon (at position 212).

Figure 5. Gel electrophoresis analysis of TNFbp/OPG chimeric polypeptides. TNFbp/OPG chimeic plasmids were transfected into CHO d-cells. supernatants from serum-free roller bottle harvests were analyzed on a 12% polyacrylamide, Tris-glycine, non-reducing gel. Dimerization patterns were compared to a TNFbp-Fc fusion (lane 1) and TNFbp monomer (lane 8).

Figure 6. Inhibition of TNFα cytotoxicity on L929 cells. Serum-free conditioned medium samples of TNFbp/Fc and TNFbp/OPG [194-401] fusion polypeptides were serially diluted and assayed for inhibition of TNFα cytotoxicity on L929 cells.

Detailed Description of the Invention

The invention provides for a chimeric polypeptide comprising a fusion of an OPG dimerization domain to a heterologous sequence.

The term "heterologous sequence" refers to an amino acid sequence which is involved in cell signalling and acts to modulate cell growth, differentiation or metabolism. In general, heterologous sequences comprise extracellular ligand binding domains of cell surface receptors and their cognate ligands. When present as part of an OPG chimeric polypeptide, a heterologous sequence of the invention comprises about ten or more amino acids in length, about 20 or more amino acids in length, about 50 or more amino acids in length, and about 100 or more amino acids in length. A heterologous sequence will be of sufficient size to confer on a chimeric polypeptide a functional property such as receptor binding, enzymatic activity, inhibitor activity and the like; however, it is understood that the chimeric polypeptides will not have functional properties identical to OPG although they may share one or more functions in common with OPG. Heterologous sequences may encode full-length polypeptides or active fragments, derivatives and analogs thereof.

In preferred embodiments, chimeric OPG polypeptides include heterologous sequences encoding growth factors, cytokines, hormones, cell adhesion molecules and other polypeptide factors which are

typically secreted. Chimeric OPG polypeptides also include heterologous sequences which encode receptors for growth factors, cytokines, hormones, cell adhesion molecules, and the like, and preferably will include extracellular ligand binding domains from said receptors, and active fragments, derivatives and analogs thereof. The heterologous sequences may or may not be capable of forming dimers or higher aggregates when the sequences are present in a naturally occurring form.

The "OPG dimerization domain" refers to that portion of the OPG polypeptide which is capable of forming covalently associated multimeric polypeptides. It is understood, however, that chimeric polypeptides comprising an OPG dimerization domain are not restricted to forming dimers, but may form higher multimers as well (trimers, tetramers, etc.) The domain may have the amino acid sequence of the human osteoprotegerein dimerization domain, or it may be a fragment, derivative or analog thereof which is capable of forming covalently associated multimers. More specifically, an OPG dimerization domain will retain one or more cysteine residues which will allow formation of at least one interchain disulfide bond. In a preferred embodiment, the OPG dimerization domain has the amino acid sequence from about residues 194 to 401 inclusive of human OPG.

As used herein, the term "fragment" comprises a deletion of one or more amino acids in a heterologous sequence or in an OPG dimerization domain. The deletion may occur at the amino terminal end, the carboxy terminal end or in an internal region of the sequence. As used herein, the term "derivative" refers to a modification of the polypeptide backbone of an OPG chimera, either within the OPG dimerization domain or within the heterologous sequence. Said modificaitons

include, but are not limited to, attachment of water soluble polymers, hydrophobic moieties, fluorescent tags, enzymatic labels and the like. As used herein, the term "analogs" refers to one or more amino acid substitutions and/or insertions within a polypeptide. Substitutions may involve conservative replacements or non-conservative replacements of amino acids which are known to one skilled in the art. Amino acid insertions may occur at the amino or carboxy terminal ends of either the OPG dimerization domain or the heterolgous sequence or both, or may occur in internal regions.

Polypeptides

Chimeric polypeptides of the invention comprise a heterologous sequence fused at its carboxy terminus to the amino terminus an OPG dimerization domain or, alternatively, an OPG dimerization domain fused at its carboxy terminus to the amino terminus of a heterologous sequence. Chimeric polypeptides may be constructed as a direct fusion of a heterologous sequence and an OPG dimerization domain or may be constructed with a spacer or adapter region having one or more amino acids inserted between the two portions of the polypeptide. Optionally, the spacer region may encode a protease cleavage site. The precise site of the fusion is not critical and may be varied by one skilled in the art in order to optimize binding charcteristics and/or biological activity of the heterologous sequence.

According to the invention, an OPG dimerization domain may be mammalian in origin (such as from mouse, rat or human) or may be a fragment or analog thereof which is capable of forming covalently associated dimers or higher order multimers. The amino acid sequences of rat, mouse and human OPG dimerization domains span from about residues 194-401 of their

respective full-length OPG polypeptides as shown in Figure 1 (SEQ ID NO:__). Fragments and analogs of an OPG dimerization domain include: deletion or substitution of a cysteine residue at any of positions 195, 202, 277, 319 and 400; addition of one or more cysteine residues; rearrangement of the configuration of cysteine residues which may entail a net increase from, a net decrease from, or no change in the number of cysteine residues compared to residues 194-401 of the human OPG dimerization domain; amino-terminal truncations of OPG[194-401], e.g, 195-401, 196-401, and so forth; C-terminal truncations of OPG[194-401], e.g, 194-400, 194-399, and so forth; conservative substitutions of amino acid residues in OPG[194-401] wherein the substitutions comprise replacements with structurally or functionally similar amino acids which are known to one skilled in the art; and any combinations thereof.

Heterologous sequences which form part of a chimeric OPG polypeptide include receptors having known extracellular ligand binding domains. Examples are receptor protein-tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR) family, fibroblast growth factor receptor (FGFR) family, insulin receptor family, epidermal growth factor receptor (EGFR) family, nerve growth factor (NGFR) family, hepatocyte growth factor family (HGFR), EPH family, AXL family, TIE family, DDR family, ROR family, and other receptor protein tyrosine kinases (see van der Geer et al. Ann. Rev. Cell Biol. 10, 251-337 (1994)). Other examples of receptors having extracellular ligand binding domains include the cytokine receptor superfamily, such as G-CSF, GM-CSF (α and β subunits), MGF, EPO, MGDF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, growth hormone, α-

interferon, β-interferon, and γ-interferon receptors, the seven transmembrane domain receptor superfamily, such as acetylcholine, adrenergic, dopamine, thrombin, FSH, gonadotropin, thyrotropoin, clacitonin and parathyroid hormone receptors, and cell adhesion receptors. It is understood that the receptors cited herein are merely examples and that heterologous sequences present in OPG chimeric polypeptides are not limited to the above-mentioned receptors.

Other heterologous sequences of the invention comprise growth factors, hormones, cytokines, cell adhesion proteins and the like. Also included are corresponding ligands for the receptor protein tyrosine kinases, ligands for cytokine receptors, ligands for seven transmembrane domain receptors, and ligands for cell adhesion receptors.

In a preferred embodiment, the heterologous sequence is a member of the TNF receptor superfamily or is derived from a member of the TNF receptor family. Members include TNFR-1, TNFR-2, TNFrp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB. Typically the extracellular domains of TNF receptors, or active fragments, derivatives and analogs thereof, are fused to an OPG dimerization domain. Active fragments of TNF receptors will have at least one cysteine rich domain, alternatively two, three or four cysteine rich domains, or alternatively one, two or three cysteine rich domains and a portion thereof, for example, two cysteine rich domains and a portion of a third domain. Activity of a TNF/OPG chimeric polypeptide may include biological activity or ligand binding activity characteristic of a TNF family member which may be evaluated using procedures known to one skilled in the art.

Preferred heterologous sequences comprise TNFR-1 or are derived from TNFR-1, and may be

a 30kDa TNF inhibitor, a 40 kDa TNF inhibitor, or a functionally active low molecular weight TNF inhibitor. The nucleic acid and amino acid sequence of mature, full-length 30kDa TNF inhibitor is shown in Figure 2 (SEQ ID NO:__). The nucleic acid and amino acid sequence of mature, full-length 40kDa TNF inhibitor is shown in Figure 3 (SEQ ID NO:__). The low molecular weight TNF inhibitors are modified forms of the 30kDa TNF inhibitor and 40 kDa TNF inhibitor which do not contain the fourth domain (amino acid residues Thr^{127}-Thr^{161} of the 30kDa TNF inhibitor and amino acid residues Pro^{141}-Thr^{179} of the 40kDa TNF inhibitor); a portion of the third domain (amino acid residues Asn^{111}-Cys^{126} of the 30kDa TNF inhibitor and amino acid residues Pro^{123}-Lys^{140} of the 40kDa TNF inhibitor); and, optionally, which do not contain a portion of the first domain (amino acid residues Asp^{1}-Lys^{21} of the 30kDa TNF inhibitor and amino acid residues Leu^{1}-Lys^{34} of the 40kDa TNF inhibitor).

The heterologous sequences of the present invention include derivatives of TNFR-1 proteins represented by the formula R_1-[Cys^{19}-Cys^{103}]-R_2 and R_4-[Cys^{32}-Cys^{112}]-R_5. These proteins are deletion variants of the 30kDa TNF inhibitor and the 40kDa TNF inhibitor, respectively, and are referred to as "truncated TNFbp(s)".

By "R_1-[Cys^{19}-Cys^{103}]-R_2" is meant one or more proteins wherein [Cys^{19}-Cys^{103}] represents residues 19 through 103 of mature, full-length 30kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 2 (SEQ ID NO:__) to facilitate the comparison; wherein R_1 represents a methionylated or nonmethionylated amine group of Cys^{19} or of amino-terminus amino acid residue(s) selected from the group:

C
IC
SIC
NSIC (SEQ ID NO:__)
NNSIC (SEQ ID NO:__)
QNNSIC (SEQ ID NO:__)
PQNNSIC (SEQ ID NO:__)
HPQNNSIC (SEQ ID NO:__)
IHPQNNSIC (SEQ ID NO:__)
YIHPQNNSIC (SEQ ID NO:__)
KYIHPQNNSIC (SEQ ID NO:__)
GKYIHPQNNSIC (SEQ ID NO:__)
QGKYIHPQNNSIC (SEQ ID NO:__)
PQGKYIHPQNNSIC (SEQ ID NO:__)
CPQGKYIHPQNNSIC (SEQ ID NO:__)
VCPQGKYIHPQNNSIC (SEQ ID NO:__)
SVCPQGKYIHPQNNSIC (SEQ ID NO:__)
DSVCPQGKYIHPQNNSIC (SEQ ID NO:__);

and wherein R_2 represents a carboxy group of Cys^{103} or of carboxy-terminal amino acid residues selected from the group:

F
FC
FCC
FCCS (SEQ ID NO:__)
FCCSL (SEQ ID NO:__)
FCCSLC (SEQ ID NO:__)
FCCSLCL (SEQ ID NO:__);

and variants thereof.

Exemplary tumor necrosis factor binding proteins which comprise TNFbp/OPG chimeric polypeptides

of the present invention include the following molecules: NH_2-MDSVCPQGKYIHPQNNSIC-[Cys^{19}-Cys^{103}]-FC-COOH (also referred to as 30kDa TNFbp 2.6C105); NH_2-MDSVCPQGKYIHPQNNSIC-[Cys^{19}-Cys^{103}]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6C106); NH_2-MDSVCPQGKYIHPQNNSIC-[Cys^{19}-Cys^{103}]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6N105); NH_2-MYIHPQNNSIC-[Cys^{19}-Cys^{103}]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d8); NH_2-M-[Cys^{19}-Cys^{103}]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d18); and NH_2-MSIS-[Cys^{19}-Cys^{103}]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d15), either methionylated or nonmethionylated, and variants and derivatives thereof.

By "R_4-[Cys^{32}-Cys^{112}]-R_5" is meant one or more proteins wherein [Cys^{32}-Cys^{112}] represents residues Cys^{32} through Cys^{112} of mature, full-length 40kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 3 (SEQ ID NO:__) to facilitate the comparison; wherein R_4 represents a methionylated or nonmethionylated amine group of Cys^{32} or of amino-terminus amino acid residue(s) selected from the group:

C
MC
QMC
AQMC (SEQ ID NO:__)
TAQMC (SEQ ID NO:__)
QTAQMC (SEQ ID NO:__)
DQTAQMC (SEQ ID NO:__)
YDQTAQMC (SEQ ID NO:__)
YYDQTAQMC (SEQ ID NO:__)
EYYDQTAQMC (SEQ ID NO:__)
REYYDQTAQMC (SEQ ID NO:__)
LREYYDQTAQMC (SEQ ID NO:__)

RLREYYDQTAQMC (SEQ ID NO:__)
CRLREYYDQTAQMC (SEQ ID NO:__)
TCRLREYYDQTAQMC (SEQ ID NO:__)
STCRLREYYDQTAQMC (SEQ ID NO:__)
GSTCRLREYYDQTAQMC (SEQ ID NO:__)
PGSTCRLREYYDQTAQMC (SEQ ID NO:__)
EPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
PEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
APEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
YAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
PYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
TPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
FTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
AFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
VAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
QVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
AQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
PAQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__)
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMC (SEQ ID NO:__);

and wherein R_5 represents a carboxy group of Cys^{112} or of carboxy-terminal amino acid residues selected from the group:

R
RL
RLC
RLCA (SEQ ID NO:__)
RLCAP (SEQ ID NO:__)
RLCAPL (SEQ ID NO:__)
RLCAPLR (SEQ ID NO:__)
RLCAPLRK (SEQ ID NO:__)
RLCAPLRKC (SEQ ID NO:__)
RLCAPLRKCR (SEQ ID NO:__)

and variants thereof.

As shown in Example 1, a hybrid DNA molecule encoding TNFbp 4.0, the full-length 30 kDa TNF inhibitor (Figure 2) with the additional sequence VKGTEDSGTT extending from the carboxy terminus, and human OPG [194-401] was constructed. The resulting chimeric polypeptide, termed TNFbp/OPG[194-401] has the amino acid sequence as shown in Figure 4. Upon expression, the mature chimeric polypeptides formed dimers in conditioned medium of transfected host cells as determined by non-reducing SDS-PAGE (see Figure 5). Additional TNFbp fusions were constructed to amino terminal truncations of the human OPG dimerization domain. These constructs are designated TNFbp/OPG[196-401], TNFbp/OPG[217-401], TNFbp/OPG[248-401], and TNFbp/OPG[304-401] and the amino acid sequences are shown in Figure 4. OPG[194-401] has the full complement of five cysteine residues which are involved in covalent association of OPG dimerization domains. OPG[196-401] lacks one cysteine residue at position 195, OPG[217-401] and OPG[248-401] lacks a second cysteine residue at position 202, and OPG[304-401] lacks a third cysteine residue at position 277 (see Figure 1 for location of cysteine residues). The chimeric polypeptides produced in conditioned medium of transfected CHOd- host cells were analyzed by non-reducing SDS-PAGE (Figure 5). In the L929 cytotoxicity assay, the TNFbp/OPG[194-401] chimera showed activity similar to a TNFbp/Fc chimera (Figure 6).

The invention also provides for chimeric OPG polypeptides which form multimers (i.e., dimers, trimers and higher multimers). Multimers of the invention comprise covalently associated monomeric OPG chimeras wherein the monomers may have identical heterologous sequence or different heterologous sequences. Preferably, the chimeric polypeptides are

dimers or trimers. Preparations of multimeric polypeptides will be essentially free of monomeric OPG chimeras which are not covalently associated and of inactive multimers. Such preparations are made using techniques available to one skilled in the art

Modifications of chimeric OPG polypeptides are encompassed by the invention and include post-translational modifications (e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of OPG which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules

will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g. EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al. Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydrl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol

molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemically modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The chimeric OPG polypeptides of the invention are isolated and purified from other constituents present in lysates or supernatants of host cells expressing the polypeptides. In one embodiment, the polypeptide is free from association with other human proteins, such as the expression product of a bacterial host cell. Also provided by the invention is a method for the purification of OPG chimeric polypeptides. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG antibody or biotin-streptavidin affinity complex and the like. When preparations of selected multimeric OPG chimeras are desired, the purification

method may be carried out to separate species of different aggregation states, for example, separation of monomeric from dimeric OPG chimeras, or separation of dimeric from tetrameric OPG chimeras.

Chimeric OPG polypeptides may be used in assays to screen for binding molecules. Examples of such molecules include, but are not limited to, nucleic acids, polypeptides, small molecular weight peptides, carbohydrates, lipids and small molecular weight organic compounds. Assays will employ combining candidate molecules (either purified or unpurified) with chimeric OPG polypeptides under conditions that allowing binding, and measuring the extent of binding to the chimeric polypeptide. Binding measurements are made using detection systems available to one skilled in the art, such as radioactivity, enzymatic activity, fluorescence, and surface plasmon resonance.

Nucleic Acids

The invention provides for an isolated nucleic acid encoding a chimeric polypeptide having an OPG dimerization domain fused to a heterologous sequence. The nucleic acids encode a chimeric OPG polypeptide wherein the heterologous sequence is a cell signalling molecule such as a receptor or a receptor ligand. In a preferred embodiment, the heterologous nucleic acid sequence encodes a polypeptide of the TNFR family, or a fragment, derivative or analog thereof, provided however that the heterologous nucleic acid sequence does not encode OPG[22-194] as shown in U.S. Serial No. 08/577,788 filed December 22, 1995, or a homologous sequence which, when fused to an OPG dimerization domain, has the biological activity of OPG.

The nucleic acids of the invention encode chimeric OPG polypeptides selected from the following:

a) the nucleic acid sequences which encode the polypeptides shown in Figure 1 (SEQ ID NO: ___) or complementary strands thereof; and

b) the nucleic acids sequences which hybridize under high stringency conditions with the sequences in (a), and degenerate sequences thereof,

provided however that the polypeptides do not have the biological activity of OPG. Nucleic acids encoding OPG chimeric polypeptides may hybridize over part or all of the nucleic acid sequences encoding the OPG dimerization domains shown in Figure 1 (SEQ ID NO: __).

The conditions for hybridization are generally of high stringency using temperatures, solvents and salt concentrations wherein the hydridizing sequences are about 12-20°C below the melting temperature (T_m) of the perfectly matched duplex. Equivalent stringency to these conditions may be readily ascertained by one skilled in the art by adjusting salt and organic solvent concentrations and temperature. Specific hybridization conditions are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

Preferred sequences include nucleic acids which encode chimeric OPG polypeptides having rat, mouse and human OPG dimerization domains. DNA encoding human OPG dimerization domain was provided in a full-length human OPG plasmid designated pRcCMV - human OPG and deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69969. DNA encoding rat OPG dimerization domain was provided in a full-length rat OPG plasmid designated pMOB-B1.1 and deposited with the American Type Culture Collection, Rockville, MD on December 27,

1995 under ATCC accession no. 69970. DNA encoding mouse OPG dimerization domain was provided in a full-length mouse OPG plasmid designated pRcCMV-murine OPG and deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69971. The nucleic acids of the invention will hybridize under stringent conditions to the DNA inserts of ATCC accession nos. 69969, 69970, and 69971.

In a preferred embodiment, heterologous sequences will comprise nucleic acids encoding TNFR-1, and fragments, derivatives and analogs thereof, such as the TNF 30kDa inhibitor or TNF 40kDa inhibitor. Presently preferred heterologous sequences include those nucleic acids encoding 30kDa TNFbp 2.6C105, 30kDa TNFbp 2.6C106, 30kDa TNFbp 2.6N105, 30kDa TNFbp 2.3d8, 30kDa TNFbp 2.3d18 and 30kDa TNFbp 2.3d15.

Also provided by the invention are nucleic acids encoding variants of an OPG chimeric polypeptide wherein the variations may be in the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives comprise addition, substitution, insertion or deletion of one or more nucleotides such that the resulting sequences encode chimeric OPG polypeptides comprising one or more amino acid residues which have been added, deleted, inserted or substituted in either the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives may be naturally occurring, such as by splice variation or polymorphism, or may be constructed using site-directed mutagenesis techniques available to the skilled worker. Chimeric OPG polypeptide variants are described in the previous section entitled "Polypeptides" and it is anticipated that nucleic acids encoding all variants disclosed therein, and degenerate molecules thereof, are encompassed by the invention.

Examples of the nucleic acids of the invention include cDNA, genomic DNA, synthetic DNA and RNA. cDNA is obtained from libraries prepared from mRNA isolated from various tissues expressing OPG. In humans, tissue sources for OPG include kidney, liver, placenta and heart. Genomic DNA encoding OPG is obtained from genomic libraries which are commercially available from a variety of species. Synthetic DNA is obtained by chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding region and flanking sequences (see U.S. Patent No. 4,695,623). RNA may be obtained in large quantities use of procaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase.

Nucleic acid sequences of the invention are useful for the expression of chimeric OPG polypeptides. Expression may be carried out in transfected host cells for production of recombinant protein in quantities sufficient for diagnostic or therapeutic applications. In addition, chimeric OPG polypeptides may be expressed in vivo and secreted into the circulation to provide therapeutic benefit.

Vectors and Host Cells

Expression vectors containing nucleic acid sequences encoding OPG fusion proteins, host cells transformed with said vectors and methods for the production of OPG fusion proteins are also provided by the invention. An overview of expression of recombinant proteins is found in Methods of Enzymology v. 185, Goeddel, D.V. ed. Academic Press (1990).

Host cells for the production of OPG fusion proteins include procaryotic host cells, such as E. coli, yeast, plant, insect and mammalian host cells.

E. coli strains such as HB101 or JM101 are suitable for expression. Preferred mammalian host cells include COS, CHOd-, 293, CV-1, 3T3, baby hamster kidney (BHK) cells and others. Mammalian host cells are preferred when post-translational modifications, such as glycosylation and polypeptide processing, are important for OPG chimera activity. Mammalian expression allows for the production of secreted polypeptides which may be recovered from the growth medium.

Vectors for the expression of OPG chimeric polypeptides contain at a minimum sequences required for vector propogation and for expression of the cloned insert. These sequences include a replication origin, selection marker, promoter, ribosome binding site, enhancer sequences, RNA splice sites and transcription termination site. Vectors suitable for expression in the aforementioned host cells are readily available and the nucleic acids of the invention are inserted into the vectors using standard recombinant DNA techniques. Vectors for tissue-specific expression of OPG chimeric polypeptides are also included. Such vectors include promoters which function specifically in liver, kidney or other organs for production in mice, and viral vectors for the expression of OPG in targeted human cells.

Using an appropriate host-vector system, OPG chimeric polypeptides are produced recombinantly by culturing a host cell transformed with an expression vector containing nucleic acid sequences encoding an OPG chimeric polypeptide under conditions such that the polypeptide is produced, and isolating the product of expression. OPG chimeras are produced in the supernatant of transfected mammalian cells or in inclusion bodies of transformed bacterial host cells. OPG chimeras so produced may be purified by procedures known to one skilled in the art as described below.

Expression vectors for mammalian hosts are exemplified by plasmids such as pDSRα described in PCT Application No. 90/14363; see also Methods in Enzymology vol. 185, D.V. Goeddel, ed. pp. 487-511 for additional examples. A variety of expression vectors are available for bacterial host cells and are described in Methods in Enzymology, ibid. pp. 14-37 and references cited therein. It is anticipated that the specific plasmids and host cells described are for illustrative purposes and that the choice of any specific plasmid and host cell for expression of an OPG chimeric polypeptide will depend upon consideration of a variety of factors by one skilled in the art.

Antibodies

Also encompassed by the invention are antibodies specifically binding to an OPG chimeric polypeptide. Antigens for the generation of antibodies may be full-length polypeptides or peptides spanning a portion of the OPG sequence. Immunological procedures for the generation of polyclonal or monoclonal antibodies reactive with OPG are known to one skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y. (1988)). Antibodies so produced are characterized for binding specificity and epitope recognition using standard enzyme-linked immunosorbent assays. Antibodies also include chimeric antibodies having variable and constant domain regions derived from different species. In one embodiment, the chimeric antibodies are humanized antibodies having murine variable domains and human constant domains. Also encompassed are complementary determining regions grafted to a human framework (so-called CDR-grafted antibodies). Chimeric

and CDR-grafted antibodies are made by recombinant methods known to one skilled in the art. Also encompassed are human antibodies made in mice.

Anti-OPG chimera antibodies of the invention may be used as an affinity reagent to purify OPG from biological samples. In one method, the antibody is immobilized on CNBr-activated Sepharose and a column of antibody-Sepharose conjugate is used to remove OPG from liquid samples. Antibodies are also used as diagnostic reagents to detect and quantitate OPG in biological samples by methods described below.

Pharmaceutical compositions

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of an OPG chimeric polypeptide together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The term "therapeutically effective amount" refers to an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascrobic acid or sodium metabisulfite. Also encompassed are compositions comprising OPG chimeric polypeptides modified with water soluble polymers to increase solubility or stability. Compositions may also comprise incorporation of OPG chimeric polypeptides into liposomes, microemulsions, micelles or vesicles for controlled delivery over an extended period of time. Selection of a particular composition will

depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of components suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

Pharmaceutical compositions of chimeric OPG polypeptides are useful for treatment of receptor-mediated disorders, for example disorders resulting from the function (or lack thereof) of protein tyrosine kinases, cytokine, seven transmembrane domain, and cell adhesion receptors. Disorders resulting from the function (or lack thereof) of the corresponding polypeptide ligands of the above referenced receptors may also be treated. In one embodiment, compositions comprising TNF/OPG chimeras are used to treat TNF-related disorders such as inflammation, autoimmune diseases and conditions marked by excessive apoptosis. Chimeras of the invention may act as agonists to stimulate receptor activation and associated changes in cell activity, or chimeras may be antagonists which block receptor function.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be

suitable for delivery to cells and tissues as part of an anti-sense or gene therapy regimen.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Construction and Expression of TNFbp/OPG fusion proteins

The TNFbp/OPG[196-401] chimeric gene was prepared in a two step PCR process. A first round of PCR was designed to produce overlapping PCR products from each gene. The templates used were plasmids p2302, containing the gene encoding TNFbp 4.0 (Figure 4) fused to the Fc region of human IgG1, and plasmid pRcCMV-human OPG (ATCC accession no. 69969), containing the gene for human OPG. The PCR products were gel purified and used as a template to create the chimeric gene. Primers used for the PCR reactions are as follows: 1275-51 (containing a 5' XbaI site, consensus Kozak and the start of the hTNFbp gene) and 1368-82 (containing a portion of OPG cDNA, an AgeI site and the 3' end of the human TNFbp 4.0 sequence) were used to amplify the TNFbp gene from p2302; 1368-83 (containing the 3' end of TNFbp, an AgeI site and the 5' end of the hOPG C-terminal domain) and 1295-27 (containing a SalI site and the 3' end of the OPG cDNA) were used to amplify the OPG[196-401] gene from pRcCMV-human OPG. A second PCR reaction used primers 1275-51 and 1295-27 to generate the chimeric gene.

The PCR product was cut with XbaI/SalI and subcloned into the pDSRα2 expression vector to give plasmid p389-1. The expression cassette contains a SV40 early promoter driving the expression of the chimeric gene and also includes an SV40 late intron, an

HTLV translation enhancing signal and an α2-FSH polyadenylation signal (DeClerck, et al. J. Biol. Chem. 266, 3893-3899 (1991)). The pDSRα2 vector also contains a DHFR cassette for selection in CHO d- cells.

Primer Sequences:

1275-51:
(SEQ ID NO:__)
5'-CGC TCTAGA CCACC ATG GGC CTC TCC ACC GTG-3'
XbaI Kozak M G L S T V

1368-82:
(SEQ ID NO:__)
5'-ACACAGGGTAACATCTAT ACCGGT GGTGCCTGAGTCCTCAG-3'
hOPG C-terminus AgeI hTNFbp

1368-83:
(SEQ ID NO:__)
5'-CTGAGGACTCAGGCACC ACCGGT ATAGATGTTACCCTGTG-3'
E D S G T T G I D V T L
TNFbp AgeI hOPG C-terminus

1295-27:
(SEQ ID NO:__)
5'-CCTCT GTCGAC TA TTA TAA GCA GCTTATTTTCACGGATTG-3'
SalI * * L C.... OPG-->

Other constructs with truncated OPG dimerization doamins were created as follows:

The primer pair for OPG[194-401] was 1295-27 and 1428-89.

1428-89:
(SEQ ID NO:__)
TCA ACCGGT AAA TGT GGA ATA GAT GTT AC
AgeI K C G I D V T

The primer pair for OPG[217-401] was 1295-27 and 1388-50.

1388-50:
(SEQ ID NO:__)
GTTT ACCGGT CCT AAC TGG CTT AGT GTC
AgeI P N W L S V

The primer pair for OPG[248-401] was 1295-27 and 1388-51.

1388-51:
(SEQ ID NO:__)
AGC ACCGGT GAA CAG ACT TTC CAG CTG
AgeI E Q T F Q L

The primer pair for OPG[304-401] was 1295-27 and 1388-52.

1388-52:
(SEQ ID NO:__)
GGAA ACCGGT CCG GGA AAG AAA GTG GG
AgeI P G K K V G

The corresponding TNFbp/OPG fusion was constructed by excising the AgeI/SalI OPG fragment from p389-1 and replacing it with AgeI/SalI digested OPG PCR products from the above reactions. The amino acid sequences encoded by the above TNFbp/OPG contructs are shown in Figure 4.

Transient transfections were performed in COS-7 cells by electroporation. Ten µg of plasmid DNA was electroporated into $2x10^6$ cells in 0.8 mls of DMEM. The electroporations were done in 0.4 cm cuvettes at 1.6 kV, 25 mF and 200 ohms. The electroporated cells were plated in 10-cm dishes in DMEM containing 10% FBS, 1x glutamine/penicillin/streptomycin, 1x non-essential amino acids, 1x Na-pyruvate. The following day the media was changed to media containing only 1% FBS. After an additional 72 hours, the conditioned media was harvested and 17 µl was electrophoresed on a 12% denaturing, non-reducing gel. These gels were blotted and analyzed by western blots for the presence of monomer and covalently-linked dimers. The primary antibody was anti-TNFbp (R&D systems, AB-225-PB) at a 1:1000 dilution and the secondary antibody was HRP, rabbit anti-goat (Pierce) at a 1:1000 dilution.

Stable transfections were done in CHO d- cells by calcium phosphate precipitation (DeClerck et al., supra). The transfection was performed as described except that 20 µg of PvuI linearized plasmid was used with 10 µg of herring sperm carrier DNA and 10 µl of calcium phosphate maximizer (Clontech) to transfect to a 10-cm dish containing approximately $5x10^5$ cells. After 2 weeks in HT- selection, colonies were ring-cloned and expanded into 24-well plates. Once confluent, two day serum-free conditioned media (SFCM) was prepared and analyzed for the expression of TNFbp/OPG fusion protein by western blot. High expressing clones were expanded and grown in roller bottles for 7d SFCM harvests. The results are shown in Figure 5.

EXAMPLE 2

Biological Activity of TNFbp/OPG chimeric proteins

WEHI Cytotoxicity Assay

The WEHI assay is an in vitro cell proliferation assay (Edwards et al. Endocrinology 128,989-996 (1991)). The cell lines are sensitive to TNF-α (i.e., TNF-α is cytotoxic). In the presence of a TNF-α inhibitor, the cells were protected from the cytotoxic effect and thus were able to proliferate.

TNF-sensitive WEHI 164 clone 13 cells are suspended at a concentration of 20 x 10^4 cells/ml in RPMI (Gibco, Grand Island, NY) medium supplemented with 5% Fetal Calf Serum (Hyclone) and penicillin 50U/ml:streptomycin 50 mg/ml. One hundred microliters of this cell suspension are placed in each well of flat-bottomed 96-cell microtiter plates, and the cells are allowed to adhere for 4-6 hours at 37°C in 7% CO_2. Medium is then aspirated, and 0.60 mg/ml actinomycin-D (Sigma Chemical Co., St. Louis, MO) is added to each well. A standard curve using serial dilutions at 0, 0.001 0.01, 0.1, 1, 10, 100 U/ml recombinant human TNF is run with each assay. Serially diluted 10-fold concentrations of TNFbp/OPG chimeras from serum-free conditioned medium are further diluted in RPMI-1640 medium containing 5% FBS and then added to duplicate wells (50 µl/well) containing adherent WEHI 164 cells after the addition of recombinant mouse TNF-α. WEHI-164 clone 13 cells are incubated for 18 hours at 37°C in 5% CO_2. Maximal killing is determined by adding 0.02% Triton X-100 (TX-100) to test wells. After incubation, 70 µl medium are aspirated, and 50 µl of a 1 mg/mL solution of the organic dye MTT tetrazolium (3-[4,5-dimethylthiozol-2-yl]2,5-diphenyl tetrazolium bromide; Sigma) is added, and cells are incubated for

an additional 4-6 hours. All supernatants are then removed, and 50 µl DMF/SDS solution (20% SDS, and 50% N,N dimethylformamide, pH 4.7) is added to each well. The DMF/SDS solution is pipetted up and down several times until all MTT crystals are dissolved, and cells were incubated for an additional 2-22 hours. The absorbances (abs) are read on a Vmax reader at 570-650. The percent specific cytotoxicity is calculated from optical densities using the formula: % specific cytotoxicity = 100% X [abs(cells + medium) - abs(cells + sample)]/abs(cells + medium) - abs(cells + TX-100)]. The number of units of TNF in each sample is determined using the percent specific cytotoxicities of the murine standards.

L929 Cytotoxicity Assay

The L929 cytotoxicity assay is an in vitro cell proliferation assay (Parmely et al. J. Immunol. 151, 389-396 (1993), the disclosure of which is hereby incorporated by reference) which also assesses the cytotoxicity of TNF-α-sensitive killing. The cell lines are sensitive to TNF-α (i.e.; TNF-α is cytotoxic). In the presence of a TNF-α inhibitor, the cells are protected from the cytotoxic effect and thus survive and are able to proliferate.

The L929 cell line was obtained from the American Type Culture Collection (catalog number ATCC CCL 1 NCTC clone 929), as described previously by Parmely et. al. (1993), supra. L929 cells were grown in tissue culture flasks in Dulbecco's MEM with 10 % fetal calf serum (FCS) to 80 % confluence. Cells were trypsinized and seeded at 8,000-10,000 cells/well in 100 ml into Falcon #3072 96 well plates and incubated for 20 to 40 hours at 37 °C in 5% CO_2. Samples of TNFbp/Fc or TNFbp/OPG [194-401] polypeptides were

serially diluted in medium and added in triplicate followed by addition of TNFα to reach a final concentration of 0.5 mg/ml. The cultures were incubated at 37 °C overnight and cell density was measured by crystal violet. Medium was removed by inverting the 96 well plates. Cells were fixed in 100 µl 100% methanol for 2 minutes. After removal of methanol the plates were allowed to dry for 10 minutes. 100 µl of 0.10% crystal violet stain in 20% methanol was added and plates were Ststained for 10 minutes at room temperature. Excess stain was removed by inverting plates. Plates were washed by dipping three times in ice-cold distilled water and excess water was removed from the wells by gently blotting plates on a tissue. 100 µl of 100% methanol was added to stained cells and optical density was measured at 595 nm. Media control reactions contained L929 cells and medium alone, and TNF control reactions contained L929 cells with 0.5 ng/ml TNFα.

The activity in this assay of TNFbp/OPG fusions constructed as described in Example 1 is shown in Figure 6.

* * *

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: CHIMERIC OPG POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 87

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-452

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asn Ser Ile Cys
1

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Gln Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

His Pro Gln Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile His Pro Gln Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
1 5 10 15

Ile Cys

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Phe Cys Cys Ser
1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Phe Cys Cys Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Phe Cys Cys Ser Leu Cys
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Phe Cys Cys Ser Leu Cys Leu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Gln Met Cys
1

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Tyr Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln
1 5 10 15

Met Cys

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala
1 5 10 15

Gln Met Cys

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr
1 5 10 15

Ala Gln Met Cys
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
1 5 10 15

Thr Ala Gln Met Cys
20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp
1 5 10 15

Gln Thr Ala Gln Met Cys
20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr
1 5 10 15

Asp Gln Thr Ala Gln Met Cys
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr
1 5 10 15

Tyr Asp Gln Thr Ala Gln Met Cys
20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu
1 5 10 15

Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg
1 5 10 15

Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu
1 5 10 15

Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg
1 5 10 15

Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys
1 5 10 15

Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr
1 5 10 15

Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25 30

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
1 5 10 15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25 30

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Arg Leu Cys Ala
1

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Arg Leu Cys Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Leu Cys Ala Pro Leu
1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Arg Leu Cys Ala Pro Leu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Arg Leu Cys Ala Pro Leu Arg Lys
1 5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Arg Leu Cys Ala Pro Leu Arg Lys Cys
1 5

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 15..32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGCTCTAGAC CACC ATG GGC CTC TCC ACC GTG 32
Met Gly Leu Ser Thr Val
1 5

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Met Gly Leu Ser Thr Val
1 5

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACACAGGGTA ACATCTATAC CGGTGGTGCC TGAGTCCTCA G 41

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CT GAG GAC TCA GGC ACC ACC GGT ATA GAT GTT ACC CTG TG 40
Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG 40

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 10..29

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCAACCGGT AAA TGT GGA ATA GAT GTT AC 29
Lys Cys Gly Ile Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Cys Gly Ile Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GTTTACCGGT CCT AAC TGG CTT AGT GTC 28
Pro Asn Trp Leu Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Asn Trp Leu Ser Val
1 5

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 10..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

AGCACCGGT GAA CAG ACT TTC CAG CTG 27
Glu Gln Thr Phe Gln Leu
1 5

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Glu Gln Thr Phe Gln Leu
1 5

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGAAACCGGT CCG GGA AAG AAA GTG GG 27
Pro Gly Lys Lys Val
1 5

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Pro Gly Lys Lys Val
1 5

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
1 5 10 15

Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp
20 25 30

Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
35 40 45

Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
50 55 60

Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp
65 70 75 80

Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His Ala Asn
85 90 95

Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro Gly Lys
100 105 110

Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Pro
115 120 125

Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
130 135 140

Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys
145 150 155 160

Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Ile
165 170 175

Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe
180 185 190

Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
195 200 205

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
1 5 10 15

Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp
20 25 30

Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
35 40 45

Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
50 55 60

Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp
65 70 75 80

Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn
85 90 95

Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys
100 105 110

Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Ser
115 120 125

Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
130 135 140

Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys
145 150 155 160

Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Met
165 170 175

Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe
180 185 190

Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
195 200 205

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```
Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
1               5                  10                  15

Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp
            20                  25                  30

Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
        35                  40                  45

Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
    50                  55                  60

Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp
65                  70                  75                  80

Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn
                85                  90                  95

Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys
            100                 105                 110

Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro
        115                 120                 125

Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
    130                 135                 140

Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys
145                 150                 155                 160

Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile
                165                 170                 175

Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe
            180                 185                 190

Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..483

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```
GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG        48
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
  1               5                  10                  15
```

```
ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT        96
Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
             20                  25                  30

CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC       144
Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
         35                  40                  45

TTC ACC GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA       192
Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
     50                  55                  60

TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC       240
Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
 65                  70                  75                  80

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG       288
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
                 85                  90                  95

AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG       336
Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
            100                 105                 110

ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC       384
Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
        115                 120                 125

CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT AAC       432
His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
    130                 135                 140

TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG       480
Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
145                 150                 155                 160

AAT                                                                   483
Asn
```

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 161 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
 1               5                  10                  15

Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
             20                  25                  30

Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
         35                  40                  45
```

Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
50 55 60

Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
65 70 75 80

Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
85 90 95

Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
100 105 110

Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
115 120 125

His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
130 135 140

Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
145 150 155 160

Asn

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 705 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..705

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

TTG CCC GCC CAG GTG GCA TTT ACA CCC TAC GCC CCG GAG CCC GGG AGC 48
Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
1 5 10 15

ACA TGC CGG CTC AGA GAA TAC TAT GAC CAG ACA GCT CAG ATG TGC TGC 96
Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys
20 25 30

AGC AAG TGC TCG CCG GGC CAA CAT GCA AAA GTC TTC TGT ACC AAG ACC 144
Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr
35 40 45

TCG GAC ACC GTG TGT GAC TCC TGT GAG GAC AGC ACA TAC ACC CAG CTC 192
Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
50 55 60

TGG AAC TGG GTT CCC GAG TGC TTG AGC TGT GGC TCC CGC TGT AGC TCT 240
Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
65 70 75 80

```
GAC CAG GTG GAA ACT CAA GCC TGC ACT CGG GAA CAG AAC CGC ATC TGC      288
Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
                 85                  90                  95

ACC TGC AGG CCC GGC TGG TAC TGC GCG CTG AGC AAG CAG GAG GGG TGC      336
Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
            100                 105                 110

CGG CTG TGC GCG CCG CTG CGC AAG TGC CGC CCG GGC TTC GGC GTG GCC      384
Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala
        115                 120                 125

AGA CCA GGA ACT GAA ACA TCA GAC GTG GTG TGC AAG CCC TGT GCC CCG      432
Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
    130                 135                 140

GGG ACG TTC TCC AAC ACG ACT TCA TCC ACG GAT ATT TGC AGG CCC CAC      480
Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
145                 150                 155                 160

CAG ATC TGT AAC GTG GTG GCC ATC CCT GGG AAT GCA AGC AGG GAT GCA      528
Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
                165                 170                 175

GTC TGC ACG TCC ACG TCC CCC ACC CGG AGT ATG GCC CCA GGG GCA GTA      576
Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
            180                 185                 190

CAC TTA CCC CAG CCA GTG TCC ACA CGA TCC CAA CAC ACG CAG CCA ACT      624
His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr
        195                 200                 205

CCA GAA CCC AGC ACT GCT CCA AGC ACC TCC TTC CTG CTC CCA ATG GGC      672
Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
    210                 215                 220

CCC AGC CCC CCA GCT GAA GGG AGC ACT GGC GAC                          705
Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

```
Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
  1               5                  10                  15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys
            20                  25                  30

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr
        35                  40                  45

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
    50                  55                  60
```

```
Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
 65                  70                  75                  80

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
                 85                  90                  95

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
            100                 105                 110

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala
        115                 120                 125

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
    130                 135                 140

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
145                 150                 155                 160

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
                165                 170                 175

Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
            180                 185                 190

His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr
        195                 200                 205

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
    210                 215                 220

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1               5                   10                  15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65                  70                  75                  80
```

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Gly Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala
210 215 220

Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser
225 230 235 240

Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val
245 250 255

Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu
260 265 270

Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys
275 280 285

Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile
290 295 300

Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser
305 310 315 320

Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys
325 330 335

Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg
340 345 350

Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu
355 360 365

Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu
370 375 380

Lys Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr
385 390 395 400

Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys
405 410 415

Ile Ser Cys Leu
420

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 211 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr
210

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 417 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1               5                  10                  15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65                  70                  75                  80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                85                  90                  95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180                 185                 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
        195                 200                 205

Gly Thr Thr Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
    210                 215                 220

Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val
225                 230                 235                 240

Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
                245                 250                 255
```

Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
260 265 270

Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln
275 280 285

Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala
290 295 300

Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly
305 310 315 320

Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys
325 330 335

Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
340 345 350

Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser
355 360 365

Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr
370 375 380

Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu
385 390 395 400

Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
405 410 415

Leu

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Gly Pro Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro
210 215 220

Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His
225 230 235 240

Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln
245 250 255

Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu
260 265 270

Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe
275 280 285

Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly
290 295 300

Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln
305 310 315 320

Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp
325 330 335

Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His
340 345 350

Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu
355 360 365

His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met
370 375 380

Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
385 390 395

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 366 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1               5                  10                  15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65                  70                  75                  80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                85                  90                  95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180                 185                 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
        195                 200                 205

Gly Thr Thr Gly Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His
    210                 215                 220

Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp
225                 230                 235                 240

Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr
                245                 250                 255
```

Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val
260 265 270

Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp
275 280 285

Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln
290 295 300

Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr
305 310 315 320

His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe
325 330 335

Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu
340 345 350

Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
355 360 365

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 311 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Gly Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys
210 215 220

Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
225 230 235 240

Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met
245 250 255

His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr
260 265 270

Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr
275 280 285

Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln
290 295 300

Ser Val Lys Ile Ser Cys Leu
305 310

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
1 5 10 15

Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
20 25 30

Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
35 40 45

Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
50 55 60

Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
65 70 75 80

Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
85 90 95

Trp Ser Glu Asn Leu Phe Gln Cys Phe Cys
100 105

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
1 5 10 15

Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
20 25 30

Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
35 40 45

Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
50 55 60

Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
65 70 75 80

Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
85 90 95

Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
100 105

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
1 5 10 15

Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
20 25 30

Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
35 40 45

Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
50 55 60

Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
65 70 75 80

Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
85 90 95

Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
100 105

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Met Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His
1 5 10 15

Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr
20 25 30

Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His
35 40 45

Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln
50 55 60

Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys
65 70 75 80

Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys
85 90 95

Phe Asn Cys Ser Leu
100

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Met Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro
1 5 10 15

Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe
20 25 30

Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys
35 40 45

Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg
50 55 60

Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser
65 70 75 80

Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
85 90

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Met Ser Ile Ser Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn
1 5 10 15

Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser
20 25 30

Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys
35 40 45

Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr
50 55 60

Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His
65 70 75 80

Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
85 90

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising an amino acid sequence of an osteoprotegerin dimerization domain fused to a heterologous amino acid sequence.

2. The polypeptide of Claim 1 wherein the heterologous amino acid sequence and the osteoprotegerin dimerization domain are human.

3. The polypeptide of Claim 1 wherein the heterologous amino acid sequence and the osteoprotegerin dimerization domain are from different species.

4. The polypeptide of Claim 1 covalently associated with one or more chimeric polypeptides which result in a mulitmeric polypeptide complex.

5. The polypeptide of Claim 4 wherein the complex is a dimer.

6. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is a membrane-bound receptor lacking functional membrane associated amino acid sequences.

7. The polypeptide of Claim 6 wherein the receptor is selected from the group consisting of receptor tryrosine kinases, cytokine receptors, seven transmembrane domain receptors, and cell adhesion receptors.

8. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is selected from members of the tumor necrosis factor-like receptor family consisting of TNFR-1, TNFR-2, TNFrp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB.

9. The polypeptide of Claim 8 wherein the heterologous sequence comprises TNFR-1 lacking functional membrane-associated sequences.

10. The polypeptide of Claim 9 wherein the heterologous sequence is a 30 kDa TNF inhibitor, a 40 kDa TNF inhibitor, or an analog thereof.

11. The polypeptide of Claim 1 wherein the carboxy terminus of the heterologous sequence is fused to the amino terminus of the OPG dimerization domain.

12. The polypeptide of Claim 1 wherein the amino terminus of the heterologous sequence is fused to the carboxy terminus of the OPG dimerization domain.

13. The polypeptide of Claim 1 wherein one or more amino acids are inserted between the heterologous sequence and the OPG dimerization domain.

14. A multimeric polypeptide comprising covalently associated monomers of OPG chimeric polypeptides.

15. The multimeric polypeptide of Claim 14 which is a dimer.

16. An isolated nucleic acid sequence encoding the polypeptide of Claim 1.

17. An expression vector comprising the nucleic acid sequence of Claim 16.

18. A host cell transformed or transfected with the expression vector of Claim 17 in a manner allowing expression of the nucleic acid.

19. A pharmaceutical composition comprising the polypeptide of any of Claims 1 to 15.

FIGURE 1

```
Rat:        Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala
Phe Phe
Mouse:  Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe
Human:  Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe

Rat:    Arg Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser
Mouse:  Arg Phe Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser
Human:  Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser

Rat:    Val Leu Val Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu
Mouse:  Val Leu Val Asp Ser Leu Pro Gly Thr Lys Val Asn Ala Glu
Human:  Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu

Rat:    Ser Val Glu Arg Ile Lys Arg Arg His Ser Ser Gln Glu Gln
Mouse:  Ser Val Glu Arg Ile Lys Arg Arg His Ser Ser Gln Glu Gln
Human:  Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln.

Rat:    Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Arg Asp
Mouse:  Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Arg Asp
Human:  Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp

Rat:    Gln Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
Mouse:  Gln Glu Met Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys
Human:  Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys

Rat:    Glu Ser Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr
Mouse:  Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn Leu Thr
Human:  Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr

Rat:    Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro Gly Lys
Mouse:  Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys
Human:  Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys

Rat:    Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys
Mouse:  Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys
Human:  Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys

Rat:    Lys Pro Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg
Mouse:  Lys Ser Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg
Human:  Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg
```

FIGURE 1 (Con't)

```
Rat:     Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr
Mouse:   Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr
Human:   Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His

Rat:     Ala Leu Lys His Leu Lys Ala Tyr His Phe Pro Lys Thr Val
Mouse:   Ala Leu Lys His Leu Lys Thr Ser His Phe Pro Lys Thr Val
Human:   Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr Val

Rat:     Thr His Ser Leu Arg Lys Thr Ile Arg Phe Leu His Ser Phe
Mouse:   Thr His Ser Leu Arg Lys Thr Met Arg Phe Leu His Ser Phe
Human:   Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe

Rat:     Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
Mouse:   Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile
Human:   Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile

Rat:     Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
Mouse:   Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
Human:   Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
```

FIGURE 2

30kDa TNF Inhibitor

```
5'-GATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAAATAATTCGATTTGCTGTACC-
   +---------+---------+---------+---------+---------+---------
   D  S  V  C  P  Q  G  K  Y  I  H  P  Q  N  N  S  I  C  C  T  -

  -AAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGGAC-
   +---------+---------+---------+---------+---------+---------
   K  C  H  K  G  T  Y  L  Y  N  D  C  P  G  P  G  Q  D  T  D  -

  -TGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACACTGCCTC-
   +---------+---------+---------+---------+---------+---------
   C  R  E  C  E  S  G  S  F  T  A  S  E  N  H  L  R  H  C  L  -

  -AGCTGCTCCAAATGCCGAAAGGAAATGGGTCAGGTGGAGATCTCTTCTTGCACAGTGGAC-
   +---------+---------+---------+---------+---------+---------
   S  C  S  K  C  R  K  E  M  G  Q  V  E  I  S  S  C  T  V  D  -

  -CGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAAAACCTT-
   +---------+---------+---------+---------+---------+---------
   R  D  T  V  C  G  C  R  K  N  Q  Y  R  H  Y  W  S  E  N  L  -

  -TTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGCCAGGAG-
   +---------+---------+---------+---------+---------+---------
   F  Q  C  F  N  C  S  L  C  L  N  G  T  V  H  L  S  C  Q  E  -

  -AAACAGAACACCGTGTGCACCTGCCATGCAGGTTTCTTTCTAAGAGAAAACGAGTGTGTC-
   +---------+---------+---------+---------+---------+---------
   K  Q  N  T  V  C  T  C  H  A  G  F  F  L  R  E  N  E  C  V  -

  -TCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAGATTGAG-
   +---------+---------+---------+---------+---------+---------
   S  C  S  N  C  K  K  S  L  E  C  T  K  L  C  L  P  Q  I  E  -

  -AAT-3'
   +-----
   N  *
```

FIGURE 3

40kDa TNF Inhibitor

```
5'-TTGCCCGCCCAGGTGGCATTTACACCCTACGCCCCGGAGCCCGGGAGCACATGCCGGCTC-
   +---------+---------+---------+---------+---------+---------
   L  P  A  Q  V  A  F  T  P  Y  A  P  E  P  G  S  T  C  R  L -

  -AGAGAATACTATGACCAGACAGCTCAGATGTGCTGCAGCAAGTGCTCGCCGGGCCAACAT-
   +---------+---------+---------+---------+---------+---------
   R  E  Y  Y  D  Q  T  A  Q  M  C  C  S  K  C  S  P  G  Q  H -

  -GCAAAAGTCTTCTGTACCAAGACCTCGGACACCGTGTGTGACTCCTGTGAGGACAGCACA-
   +---------+---------+---------+---------+---------+---------
   A  K  V  F  C  T  K  T  S  D  T  V  C  D  S  C  E  D  S  T -

  -TACACCCAGCTCTGGAACTGGGTTCCCGAGTGCTTGAGCTGTGGCTCCCGCTGTAGCTCT-
   +---------+---------+---------+---------+---------+---------
   Y  T  Q  L  W  N  W  V  P  E  C  L  S  C  G  S  R  C  S  S -

  -GACCAGGTGGAAACTCAAGCCTGCACTCGGGAACAGAACCGCATCTGCACCTGCAGGCCC-
   +---------+---------+---------+---------+---------+---------
   D  Q  V  E  T  Q  A  C  T  R  E  Q  N  R  I  C  T  C  R  P -

  -GGCTGGTACTGCGCGCTGAGCAAGCAGGAGGGGTGCCGGCTGTGCGCGCCGCTGCGCAAG-
   +---------+---------+---------+---------+---------+---------
   G  W  Y  C  A  L  S  K  Q  E  G  C  R  L  C  A  P  L  R  K -

  -TGCCGCCCGGGCTTCGGCGTGGCCAGACCAGGAACTGAAACATCAGACGTGGTGTGCAAG-
   +---------+---------+---------+---------+---------+---------
   C  R  P  G  F  G  V  A  R  P  G  T  E  T  S  D  V  V  C  K -

  -CCCTGTGCCCCGGGGACGTTCTCCAACACGACTTCATCCACGGATATTTGCAGGCCCCAC-
   +---------+---------+---------+---------+---------+---------
   P  C  A  P  G  T  F  S  N  T  T  S  S  T  D  I  C  R  P  H -

  -CAGATCTGTAACGTGGTGGCCATCCCTGGGAATGCAAGCAGGGATGCAGTCTGCACGTCC-
   +---------+---------+---------+---------+---------+---------
   Q  I  C  N  V  V  A  I  P  G  N  A  S  R  D  A  V  C  T  S -

  -ACGTCCCCCACCCGGAGTATGGCCCCAGGGGCAGTACACTTACCCCAGCCAGTGTCCACA-
   +---------+---------+---------+---------+---------+---------
   T  S  P  T  R  S  M  A  P  G  A  V  H  L  P  Q  P  V  S  T -

  -CGATCCCAACACACGCAGCCAACTCCAGAACCCAGCACTGCTCCAAGCACCTCCTTCCTG-
   +---------+---------+---------+---------+---------+---------
   R  S  Q  H  T  Q  P  T  P  E  P  S  T  A  P  S  T  S  F  L -

  -CTCCCAATGGGCCCCAGCCCCCCAGCTGAAGGGAGCACTGGCGAC-3'
   +---------+---------+---------+---------+---------+---------
   L  P  M  G  P  S  P  P  A  E  G  S  T  G  D  *
```

FIGURE 4

```
          1                                                    50
TNFbp/OPG MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI
TNFbp 4.0 MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI
TNFbp/196 MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI
TNFbp/217 MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI
TNFbp/248 MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI
TNFbp/304 MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR DSVCPQGKYI

          51                                                  100
TNFbp/OPG HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL
TNFbp 4.0 HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL
TNFbp/196 HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL
TNFbp/217 HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL
TNFbp/248 HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL
TNFbp/304 HPQNNSICCT KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL

          101                                                 150
TNFbp/OPG SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL
TNFbp 4.0 SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL
TNFbp/196 SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL
TNFbp/217 SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL
TNFbp/248 SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL
TNFbp/304 SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL

          151                                                 200
TNFbp/OPG NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE
TNFbp 4.0 NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE
TNFbp/196 NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE
TNFbp/217 NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE
TNFbp/248 NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE
TNFbp/304 NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE

          201                                                 250
TNFbp/OPG NVKGTEDSGT TGKCGIDVTL CEEAFFRFAV PTKFTPNWLS VLVDNLPGTK
TNFbp 4.0 NVKGTEDSGT T......... .......... .......... ..........
TNFbp/196 NVKGTEDSGT T...GIDVTL CEEAFFRFAV PTKFTPNWLS VLVDNLPGTK
TNFbp/217 NVKGTEDSGT TG........ .......... .....PNWLS VLVDNLPGTK
TNFbp/248 NVKGTEDSGT TG........ .......... .......... ..........
TNFbp/304 NVKGTEDSGT TG........ .......... .......... ..........
                          196(OPG)                217(OPG)

          251                                                 300
TNFbp/OPG VNAESVERIK RQHSSQEQTF QLLKLWKHQN KDQDIVKKII QDIDLCENSV
TNFbp 4.0 .......... .......... .......... .......... ..........
TNFbp/196 VNAESVERIK RQHSSQEQTF QLLKLWKHQN KDQDIVKKII QDIDLCENSV
TNFbp/217 VNAESVERIK RQHSSQEQTF QLLKLWKHQN KDQDIVKKII QDIDLCENSV
TNFbp/248 .......... ......EQTF QLLKLWKHQN KDQDIVKKII QDIDLCENSV
TNFbp/304.......... .......... .......... .......... ..........
                               248(OPG)
```

```
          301                                                        350
TNFbp/OPG QRHIGHANLT FEQLRSLMES LPGKKVGAED IEKTIKACKP SDQILKLLSL
TNFbp 4.0 .......... .......... .......... .......... ..........
TNFbp/196 QRHIGHANLT FEQLRSLMES LPGKKVGAED IEKTIKACKP SDQILKLLSL
TNFbp/217 QRHIGHANLT FEQLRSLMES LPGKKVGAED IEKTIKACKP SDQILKLLSL
TNFbp/248 QRHIGHANLT FEQLRSLMES LPGKKVGAED IEKTIKACKP SDQILKLLSL
TNFbp/304 .......... .......... .PGKKVGAED IEKTIKACKP SDQILKLLSL
                                  304(OPG)
          351                                                        400
TNFbp/OPG WRIKNGDQDT LKGLMHALKH SKTYHFPKTV TQSLKKTIRF LHSFTMYKLY
TNFbp 4.0 .......... .......... .......... .......... ..........
TNFbp/196 WRIKNGDQDT LKGLMHALKH SKTYHFPKTV TQSLKKTIRF LHSFTMYKLY
TNFbp/217 WRIKNGDQDT LKGLMHALKH SKTYHFPKTV TQSLKKTIRF LHSFTMYKLY
TNFbp/248 WRIKNGDQDT LKGLMHALKH SKTYHFPKTV TQSLKKTIRF LHSFTMYKLY
TNFbp/304 WRIKNGDQDT LKGLMHALKH SKTYHFPKTV TQSLKKTIRF LHSFTMYKLY

          401                 420
TNFbp/OPG QKLFLEMIGN QVQSVKISCL
TNFbp 4.0 .......... ..........
TNFbp/196 QKLFLEMIGN QVQSVKISCL
TNFbp/217 QKLFLEMIGN QVQSVKISCL
TNFbp/248 QKLFLEMIGN QVQSVKISCL
TNFbp/304 QKLFLEMIGN QVQSVKISCL
                                  401(OPG)
```

FIGURE 5

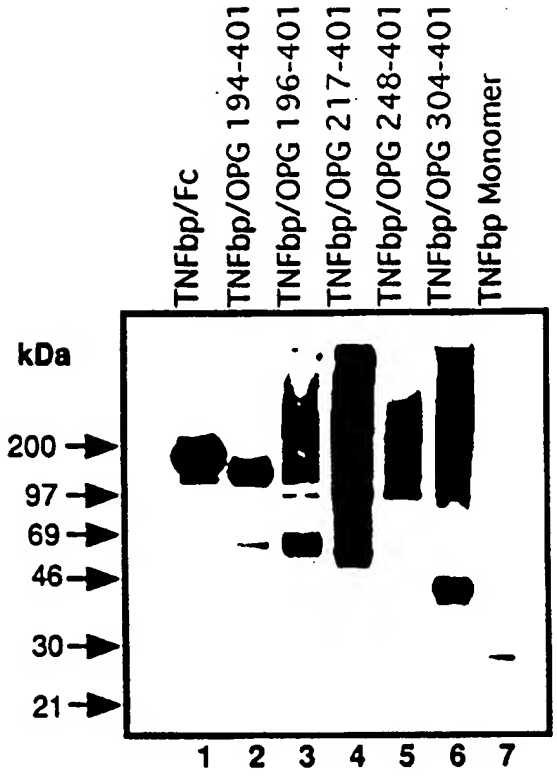
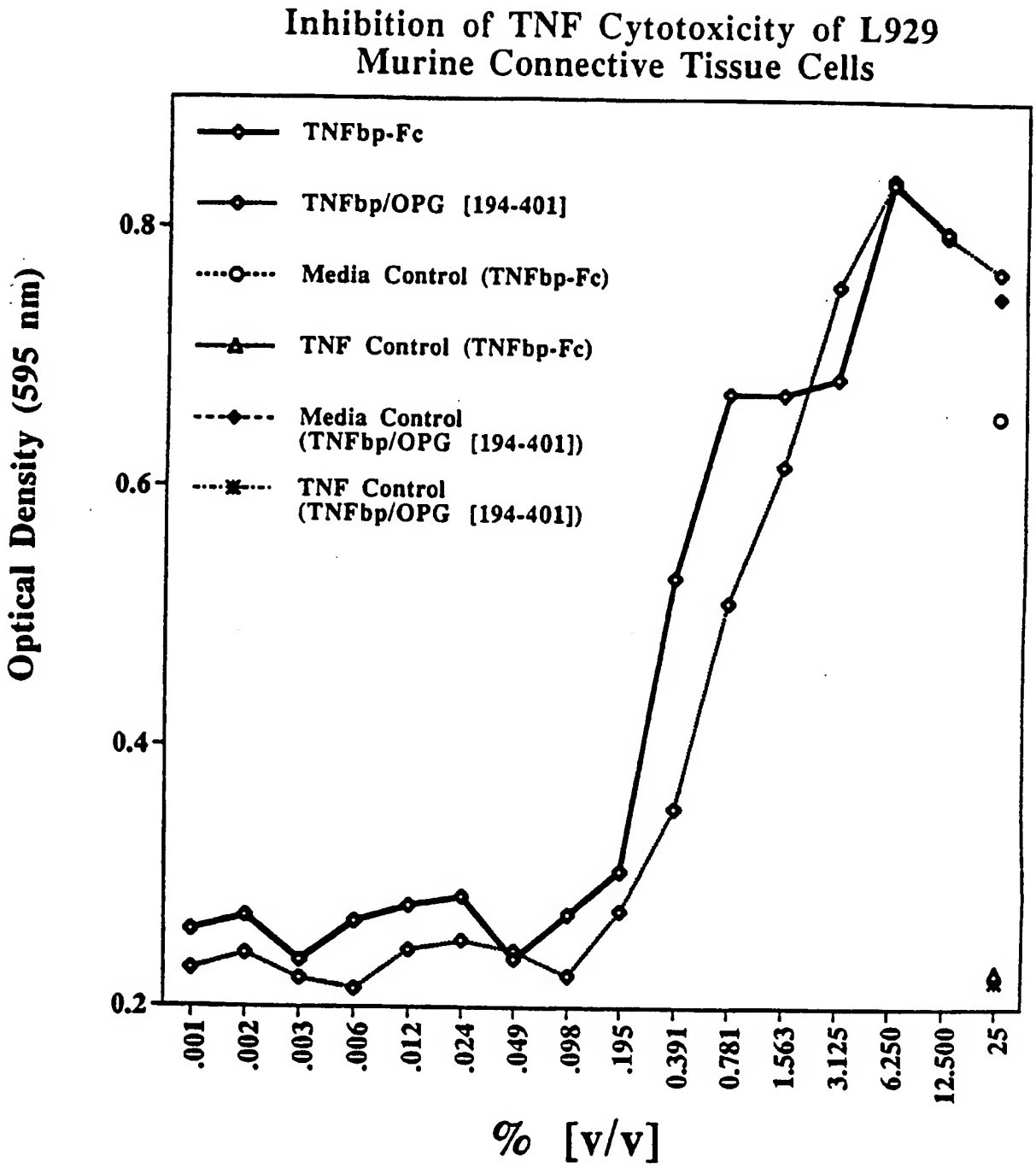

TNFbp/Fc
TNFbp/OPG 194-401
TNFbp/OPG 196-401
TNFbp/OPG 217-401
TNFbp/OPG 248-401
TNFbp/OPG 304-401
TNFbp Monomer
kDa
200
97
69
46
30
21
1 2 3 4 5 6 7

FIGURE 6


Inhibition of TNF Cytotoxicity of L929
Murine Connective Tissue Cells
TNFbp-Fc
TNFbp/OPG [194-401]
Media Control (TNFbp-Fc)
TNF Control (TNFbp-Fc)
Media Control
(TNFbp/OPG [194-401])
TNF Control
(TNFbp/OPG [194-401])
Optical Density (595 nm)
0.8
0.6
0.4
0.2
.001
.002
.003
.006
.012
.024
.049
.098
.195
0.391
0.781
1.563
3.125
6.250
12.500
25
% [v/v]

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 98/08631

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C07K14/705 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	EP 0 816 380 A (SNOW BRAND MILK PROD CO LTD) 7 January 1998 See, in particular, the OCIF-C23S,-CL and -CC mutants in example 22.	1-19
Y	& WO 96 26217 A (SNOW BRAND MILK PRODUCTS) 29 August 1996 ---	1-19
Y	EP 0 526 905 A (YEDA RES & DEV) 10 February 1993 See, in particular, example 3d. ---	1-19
P,X	WO 97 23614 A (AMGEN INC ;BOYLE WILLIAM J (US); LACEY DAVID L (US); CALZONE FRANK) 3 July 1997 See examples 8.U and 9 --- -/--	1-3, 11-13, 16-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 September 1998	25/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 98/08631

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	YAMAGUCHI K ET AL: "Characterisation of structural domains of human osteoclastogenesis inhibitory factor" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 273, no. 9, 27 February 1998, pages 5117-5123, XP002077021	

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat' Application No
PCT/US 98/08631

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0816380	A	07-01-1998	AU	4677396 A	11-09-1996
			FI	973402 A	17-10-1997
			NO	973801 A	20-10-1997
			CA	2213469 A	29-08-1996
			CN	1175956 A	11-03-1998
			WO	9626217 A	29-08-1996
EP 0526905	A	10-02-1993	AU	661008 B	13-07-1995
			AU	2090992 A	11-02-1993
			CA	2075358 A	08-02-1993
			JP	7145068 A	06-06-1995
			US	5478925 A	26-12-1995
			ZA	9205904 A	25-03-1993
WO 9723614	A	03-07-1997	AU	1468697 A	17-07-1997
			CA	2210467 A	03-07-1997
			CN	1182452 A	20-05-1998
			DE	19654610 A	26-06-1997
			EP	0784093 A	16-07-1997
			FR	2742767 A	27-06-1997
			GB	2312899 A	12-11-1997
			NO	973699 A	21-10-1997
			PL	321938 A	05-01-1998